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Original Article

INFLUENCE OF STORAGE ON CHEMICAL AND MICROBIOLOGICAL ATTRIBUTES OF FOXTAIL MILLET (*SETARIA ITALICA*) FLOUR INCORPORATED COMPLEMENTARY FOOD

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ABSTRACT

A complementary food was formulated by dry blending of malted foxtail millet (Setaria italica) and wheat flours at 30 and 40 parts, skimmed milk powder 30 parts, ghee 7 parts, sugar 12 parts and 2 per cent whey protein concentrate (CF_1) and another without WPC (CF_2). A wheat based Commercial complementary food was used as control. To study the influence of storage on chemical and microbiological attributes all the complementary foods packed in separate low-density polyethylene pouches and stored at 27±2ºC for 60 days. The influence of storage on the chemical parameters viz., per cent FFA, per cent soluble nitrogen and HMF determined at every 15 days interval of time showed gradual increase in these parameters from 0.24 to 1.83 and 0.26 to 1.92 percent oleic acid, 0.21 to 2.96 and 0.24 to 3.08 per cent soluble nitrogen, 9.4 to 17.96 and 9.56 to 18.24 µmole/ 100g for CF_1 and CF_2 respectively. The total bacterial counts increased from 3.39 to 4.05 and 3.44 to 4.12 log_{10}cfu per gram for CF_1 and CF_2 respectively. The yeast and molds were absent until 45th day of storage and appeared on the 60th day, whereas the coliforms were absent in all the three complementary foods throughout the storage period. Hence, the foxtail millet based complementary food, developed may be stored for 45 days without any significant deterioration which can still have the microbial counts within the prescribed legal standards and safe for consumption.

KEYWORDS: Complementary Food, Foxtail Millet, Free Fatty Acids, Proteolysis and Hydroxy Methyl Furfural

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INTRODUCTION

The storage of designed food plays a dominant role in deciding the effectiveness and sustainability of food formulations. Moisture and relative humidity are the critical factors, which influence the storage stability of the product[1]. The quality of any food deteriorates during storage due to the biochemical changes that takes place. The shelf life of a food is an important parameter, which determines the potential utilization of the food commodity. During storage of complementary food several physical, chemical and microbiological changes takes place, which may decrease the consumer acceptability and render the food unsafe for consumption. The important chemical changes are due to lipolysis, proteolysis and maillard browning reactions in addition to microbial growth. Hence, in the present investigation an attempt was made to evaluate the chemical and microbiological changes to explore the keeping quality of the complementary foods that meet the requirements of [2], prepared with malted foxtail millet (*Setaria italica*), wheat flour and milk solids with and without WPC.

MATERIALS AND METHODS

The raw materials for the complementary food viz., foxtail millet, wheat, sugar and Nandini brand ghee and skim milk powder (Nandini) were obtained from local market (Bengaluru) and whey protein concentrate (80%) was obtained from Mahaan Protein Limited suppliers (Delhi).

Complementary Food Formulation

The complementary food was developed after malting the foxtail millet (*Setaria italica*), and wheat, thus obtained malted flours were taken in the proportion of 30:40 to which 30 parts skimmed milk powder, 7 parts ghee, 12 parts sugar and 2 parts of WPC were added (CF_2). Another complementary food without WPC (CF_1) was also formulated. Commercially available wheat based complementary food was used as control.

Storage Stability of Complementary Food

The complementary food were packed in LDPE polyethylene pouches separately, stored at 27±2ºC for a period of 60 days. The predominant chemical changes that takes place during storage like per cent FFA by [3], per cent soluble nitrogen as per [4] and HMF (browning index) by [5] were analyzed on 0, 15, 30, 45 and 60th day of storage resoectively.

Microbiological Analysis

The stored samples of complementary foods were subjected for enumeration of TBC as per [6], Coliform count by [7], Yeast and Molds by [8] count for a period of 60 days at an interval of 15 days in order to declare the acceptability of the product.

Statistical Analysis

The data obtained in the research work was analyzed using One way ANOVA using R software (R. version 3.1.3(2015-03-09). Copyright (C) 2015.

RESULTS AND DISCUSSIONS

During the storage period, there were no conspicuous physical changes. However, gradual chemical changes in the samples were analyzed.

Free Fatty Acids (% FFA)

It is evident from the table 1 that, upon storage there was a gradual increase in the FFA content. Although the FFA levels on the 0th day in all the complementary foods was not significant, it increased throughout the storage period. The FFA levels were higher for CF_1 and CF_2 when compared to the control complementary food. For CF_1 and CF_2 the FFA levels increased from 0.24 to 1.83 and 0.26 to 1.92 per cent oleic acid respectively, whereas for the control it varied from 0.23 to 1.52 per cent oleic acid. This might be due to the addition of ghee at higher levels (7 per cent) and lipolytic activity of the microorganisms. Besides, the germination of millet grains results in the hydrolysis of lipids and oxidation of fatty acids[9]. At the 60th day of storage the stored complementary food with and without WPC showed FFA levels of 1.83 and 1.92 per cent oleic acid respectively. Similar results were reported by [10] during the storage of weaning food developed for milk allergic children. In order to minimize the levels of FFA, ghee may be added during the time of preparation of the gruel, instead of adding before.

Proteolytic Changes (% Soluble Nitrogen)

It is evident from the table 2 that upon storage there was an increase in the soluble nitrogen content. At 0^{th} day, the control sample and the experimental complementary food showed soluble nitrogen content of 0.16, 0.21 and 0.24 per cent respectively. Between the experimental complementary foods, CF_1 and CF_2, the CF_2 sample was observed to contain higher soluble nitrogen content than CF_1. The soluble nitrogen content of CF_1 and CF_2 increased from 0.21 to 2.96 and 0.24 to 3.08 per cent, respectively. This increase in soluble nitrogen might be due to the proteolytic activity of the bacteria and a small increase in moisture content of the stored complementary food.

Hydroxy Methyl Furfural (HMF)

HMF that indicates browning effect during storage is shown in table 3. There was an increase in the HMF content throughout the storage period. The HMF content was higher in case of experimental samples when compared to the control. In the control sample the HMF content varied from 10.08 to 19.21µmole/ 100g, whereas for CF_1 and CF_2 the HMF content ranged between 9.40 to 17.96µmole/ 100g and 9.56 to 18.24µmole/ 100g respectively. This increase in HMF content might be due to increased absorption of moisture by the foxtail millet flour and increased soluble nitrogen. Similar increase in the HMF content from 9.12 to 14.38µmole/100g in milk based infant formula was reported by[11].

Microbiological Quality of the Stored Complementary Foods

As per the recommendation of [2], the total bacterial count should not exceed 10,000 cfu/g (4.00 $\log_{10}$ cfu/g) and the coliforms and yeast and molds should be absent in 0.1g of sample (absent in first dilution). The observed TBC value during 0^{th} day in the present study were 3.21, 3.39 and 3.44 $\log_{10}$ cfu/g for control, CF_1 and CF_2. At the end of 60 days TBC value was 4.03, 4.05 and 4.12 $\log_{10}$ cfu/g for control, CF_1 and CF_2 respectively. The initial count of TBC in the product was due to the ingredients such as wheat and foxtail millet flours, sugar and WPC, which were having lower water activity, might have lead to the development of bacterial spores and molds. The counts were within the permissible limits of [2], standards up to 45^{th} day of storage. Later increase in the counts might be attributed to absorption of moisture from the environment thereby increasing the water activity resulting in higher TBC counts. There was no presence of coliforms in complementary foods throughout the storage period. The yeast and mold counts were absent till 45^{th} day and their presence was observed at the 60^{th} day of storage. Yeast and mold counts appearing on 60^{th} day can be attributed to the fact that they might be present in lower number and took longer adjustment period to exhibit on selective medium. Once they form colony, such complementary food cannot be fed to the infants due to the risk of mycotoxins production from molds, which causes health hazards. Keeping in view of the microbial standards, the product can be stored safely up to 45days at household levels when stored in polyethylene pouches at ambient temperature.

CONCLUSIONS

From the present study, it may be concluded that the CF_1 and CF_2 may be safe for consumption until 45 days of storage at 27±2°C**.** To enhance the shelf life of the product it must be packed in multiple laminates with inert or gas packaging or addition of milk fat may be done at the time of using. The use of packaging materials like PP-BOPP (polypropylene -biaxially oriented polypropylene) or metalized polyester which have relatively stronger barrier properties is recommended for enhancement of storage stability and safe commercial exploitation.

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APPENDICES

Table 1: Effect of Storage on Free Fatty Acid Content* of the Complementary Foods (27±2°C)

Type of CF	Storage Period (Days) Free Fatty Acids (% oleic acid)				
	0	**15**	**30**	**45**	**60**
Control	0.23	0.41	0.74	1.08	1.52
CF_1	0.24	0.45	0.81	1.24	1.83
CF_2	0.26	0.51	0.94	1.36	1.92
CD ($P \leq 0.05$)	NS	0.04	0.036	0.04	0.03

All values are average of three trials

CD- Critical difference

*Expressed as percent Oleic acid.

NS – Non-Significant

CF – Complementary food

Control: Commercial sample

CF_1 – Foxtail millet malt: Wheat malt (30: 40) + 30 parts SMP + 7 parts ghee + 12% sugar

CF_2 – Foxtail millet malt: Wheat malt (30: 40) + 30 parts SMP + 7 parts ghee + 2%WPC+ 12% sugar.

Table 2: Effect of Storage on Proteolytic Changes (% Soluble Nitrogen) of the Complementary Foods

Type of CF	Storage Period (Days) (% Soluble Nitrogen)				
	0	15	30	45	60
Control	0.16	0.63	1.08	1.79	2.64
CF_1	0.21	0.86	1.32	2.11	2.96
CF_2	0.24	1.02	1.49	2.32	3.08
CD (P≤0.05)	NS	0.04	0.039	0.028	0.03

All values are average of three trials

CD- Critical difference

*Expressed as percent soluble nitrogen

NS – Non-Significant

CF – Complementary food

Control: Commercial sample

CF_1 – Foxtail millet malt: Wheat malt (30: 40) + 30 parts SMP + 7 parts ghee + 12% sugar

CF_2 – Foxtail millet malt: Wheat malt (30: 40) + 30 parts SMP + 7 parts ghee + 2% WPC+ 12% sugar.

Table 3: Effect of Storage on Hydroxy Methyl Furfural (HMF) Content of Complementary Foods

Type of CF	Storage Period (Days) HMF (µ mole/ 100g)				
	0	15	30	45	60
Control	10.08	11.16	14.03	16.54	19.21
CF_1	9.40	10.82	13.26	15.02	17.96
CF_2	9.56	11.04	13.80	15.63	18.24
CD (P≤0.05)	0.23	0.04	0.039	0.026	0.034

All values are average of three trials

CD- Critical difference

*Expressed as µ mole/ 100g

CF – Complementary food

Control: Commercial sample

CF_1 – Foxtail millet malt: Wheat malt (30: 40) + 30 parts SMP + 7 parts ghee + 12% sugar

CF_2 – Foxtail millet malt: Wheat malt (30: 40) + 30 parts SMP + 7 parts ghee + 2% WPC+ 12% sugar

Table 4: Effect of Storage on Microbial Quality of Complementary Foods

Type of CF		Storage Period (Days)				
		0	15	30	45	60
Control	TBC	3.21	3.42	3.60	3.75	4.03
	Coliforms	0.00	0.00	0.00	0.00	0.00
	Yeast and Molds	0.00	0.00	0.00	0.00	0.00
CF_1	TBC	3.39	3.53	3.64	3.76	4.05
	Coliforms	0.00	0.00	0.00	0.00	0.00

	Table 4: Contd.,					
	Yeast and Molds	0.00	0.00	0.00	0.00	1.48
CF_2	TBC	3.44	3.56	3.66	3.79	4.12
	Coliforms	0.00	0.00	0.00	0.00	0.00
	Yeast and Molds	0.00	0.00	0.00	0.00	1.60

All values are average of three trials

CD- Critical difference

*Expressed as $\log_{10}$cfu per gram

CF – Complementary food

Control: Commercial sample

CF_1 – Foxtail millet malt: Wheat malt (30: 40) + 30 parts SMP + 7 parts ghee + 12% sugar

CF_2 – Foxtail millet malt: Wheat malt (30: 40) + 30 parts SMP + 7 parts ghee + 2% WPC+ 12% sugar